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**13. ABSTRACT (Maximum 200 Words)**

The overall goal of this research is to determine if human breast cancer can be prevented from becoming angiogenic when it is still at a microscopic size of less than approximately 1 $mm^3$. We have made the following progress during the past year: (1) We have developed models in SCID mice of four different non-angiogenic human breast cancers, and have shown that the time to the switch to the angiogenic phenotype is predictable and reproducible for each different breast cancer type. The same is true for the percentage of tumors that become angiogenic. (2) Two angiogenesis-based biomarkers have been developed to detect the angiogenic switch when these tumors are still at a microscopic size of 1 $mm^3$ or less. The most sensitive and accurate biomarker is the "platelet angiogenic profile," which determines and quantifies the angiogenic regulatory proteins being elaborated by a tumor. A second biomarker is a rise in circulating precursor endothelial cells exiting from the bone marrow and stimulated by a tumor that is undergoing the angiogenic switch.

Our translational goal is to treat human breast cancer, both primary and recurrent, with non-toxic angiogenesis inhibitors guided by biomarkers before tumors can be anatomically located.

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## Table of Contents

Innovator Award Progress Report
P.I. Moses Judah Folkman, M.D. (Judah Folkman)

**PREVENTION OF THE ANGIOGENIC SWITCH IN HUMAN BREAST CANCER.**

## I. INTRODUCTION:

The purpose of this research is to determine if human breast cancer (and other human tumors) can be prevented from becoming angiogenic when they are still at a microscopic size of less than approximately 1 millimeter diameter. We have proposed to develop angiogenesis-based biomarkers that will recognize the presence of such an early tumor long before it could be diagnosed by any conventional methods such as magnetic resonance imaging, CAT scan, or ultrasound. We have further proposed to study the mechanism of the switch to the angiogenic phenotype. We wish to prevent the angiogenic switch in human breast cancer by long-term administration of non-toxic angiogenesis inhibitors, or by increasing the level of one or more endogenous angiogenesis inhibitors in the circulation by oral administration of non-toxic drugs which increase the expression of these angiogenesis inhibitors.

The translational goal of this project is two-fold: (i) For women who have had a primary breast cancer surgically removed or treated by another modality, angiogenesis-based biomarkers in the blood and urine will be quantified periodically (every few months), in the post-operative period. These biomarkers will be developed during the support of this Innovator Award. They will include the platelet angiogenic profile and circulating endothelial precursor cells. (Urinary metalloproteinases will be used in conjunction with these two biomarkers and to validate them. Marsha Moses, Ph.D., in our laboratory will collaborate with Dr. Folkman on this aspect of the project). Based on what we have learned from mouse studies so far, women whose biomarkers begin to rise in the post-operative period, would have a high risk of recurrence several years before such a recurrence could be predicted with today's technology. These women would be offered treatment by relatively non-toxic antiangiogenic therapy until the biomarkers returned to normal and for a given period beyond that time. Angiogenesis-based biomarkers would continue to be tested periodically as a surveillance measure.

(ii) Women with the mutated breast cancer gene may be monitored by periodic tests of angiogenesis-based biomarkers. Rising biomarkers could be treated as described above. If the biomarkers fell to normal, this could possibly obviate the current medical practice of advising these women to have bilateral mastectomy and oophorectomy. These angiogenesis based biomarker blood and urine tests will need to be validated in clinical trials. Our goal during the period of support of this Innovator Award, is to complete the development of

three angiogenesis-based biomarkers and to initiate the clinical trials for validation. We estimate that approximately 2-3 years would be needed to validate these three tests in patients by using all of the Harvard Hospitals.

We also envisage that the results from these studies will apply generally to other tumors, for example colon cancer and prostate cancer.

## II. BODY:

Significant progress has been made in all three tasks.

**Task 1. Median time of switching to the angiogenic phenotype for human breast cancers.** (Judah Folkman, M.D., Yuen Shing, Ph.D., George Naumov, Ph.D., Deborah Freedman, Ph.D. & Gang Liang, Ph.D.)

We have previously characterized the subcutaneous tumor growth of both angiogenic and non-angiogenic tumor cells from a human breast cancer cell line (MDA-MB-436). When SCID immunodeficient mice were inoculated subcutaneously with human breast cancer cells that were either non-angiogenic or angiogenic, tumors first became reliably palpable at approximately 50 $mm^3$. The mean time to palpation after inoculation of angiogenic breast cancer cells was 19 days (mean, 95% confidence interval [CI] =16-22 days), and in mice injected with the non-angiogenic cells was 119 days (mean, 95% CI= 53-185 days).

We have found that angiogenic tumors virtually always contain sub-populations of non-angiogenic tumor cells. We separated non-angiogenic tumor cells from angiogenic tumor cells of a human breast cancer (MDA-MB-436) and cloned a single non-angiogenic tumor cell (called clone A1). Tumor cells from clone A1 remained non-angiogenic for a mean of 234 days, (95% CI=199-269 days). Approximately 5-10% of implanted tumors from A1 switched to the angiogenic phenotype.

The non-angiogenic and angiogenic tumor cells had equivalent proliferation rates *in vitro*. Nevertheless, we found that even when a single tumor cell was cloned from an angiogenic tumor, expanded in cell culture, and inoculated into a mouse to produce an angiogenic tumor, the majority of the cells produced angiogenic tumors which grew rapidly. However, a few cells from the expanded angiogenic clone were found to be non-angiogenic.

The reversion of some angiogenic tumor cells to non-angiogenic tumor cells was only recently discovered in my laboratory during support from the Innovator Award, but the mechanism of this reversion remains unknown. It is one of the interesting problems that we will try to attack during the coming year.

At day 350 after injection of the human breast cancer cells, the cumulative incidence of palpable tumors was as follows: mice inoculated with angiogenic cells, 100%; with mice inoculated with non-angiogenic cells, 50-80%; and with non-angiogenic clone A1 cells, 10-20%.

Orthotopic inoculation of breast cancer cells into the mammary fat pad gave similar results, except that in the orthotopic site the switch to the angiogenic phenotype was delayed by approximately 30 days when compared to subcutaneously grown tumors.

We have infected all three sub-populations with Luciferase for more quantitative *in vivo* tumor growth monitoring. In addition, we have found that the Luciferase signal reveals a sharp increase in light flux (the angiogenic switch), approximately 20 days before the appearance of a palpable tumor.

In addition to the detailed characterization of human breast cancer cell lines that produce dormant tumors (MDA-MB-436), we are currently characterizing three other breast cancer cell lines: MDA-MB-415, MDA-MB-435S, and MDA-MB-453 (see Fig.1). When injected subcutaneously into mice MDA-MB-415 human breast cancer cells remained non-angiogenic and dormant at a

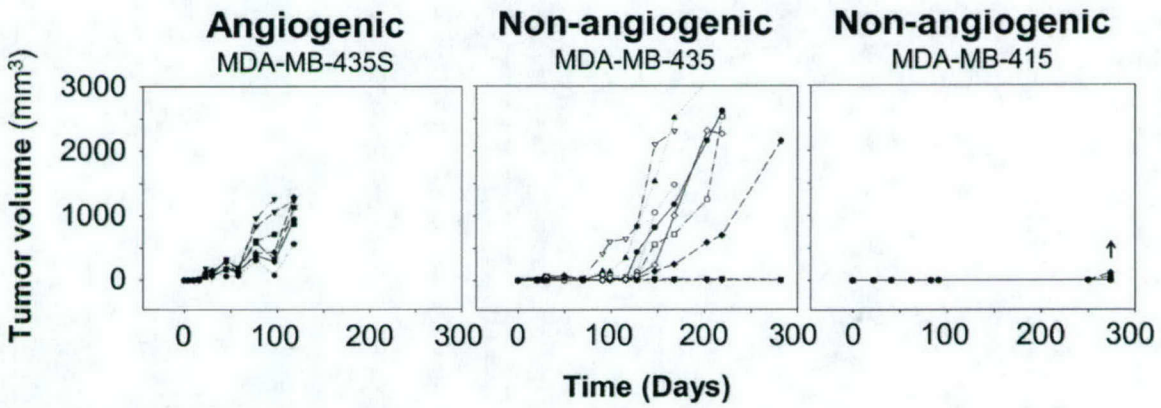



**Figure 1:** Human breast cancer cell lines in SCID mice. $5x10^6$ tumor cells implanted subcutaneously. Left panel: shows angiogenic cells cloned from a single cell. Middle panel: non-angiogenic cells from a different tumor line cloned from a single cell showing the angiogenic switch in approximately 70% of tumors at a median of approximately 120 days. Right panel: non-angiogenic cells from a different breast cancer cell line. Dormant tumors for up to 250 days. One tumor has begun the angiogenic switch at 275 days (this is close to the normal lifetime of these SCID mice).

microscopic size (< $1mm^3$) for up to 280 days after injection. Currently, 2 out of 8 mice have palpable tumors which are less than 100 $mm^3$ in size. Thus, the initial observations from the MDA-MB-436 tumor cells, also appear to be reproducible for a different human breast cancer cell line (i.e., MDA-MB-415). All mice injected with the MDA-MB-453 breast (angiogenic) cancer cells formed palpable tumors

30 days after inoculation. However these tumors grew very slowly over the next 220 days to a final size of approximately 1700 $mm^3$. Similar slow tumor growth was observed when MDA-MB-435S cells were injected subcutaneously. All mice inoculated with these cells developed palpable tumors by day 10 and formed large tumors by day 280 after injection.

We have developed two cell lines from two MDA-MB-453 tumors. When mice were re-inoculated with the angiogenic sub-population of the MDA-MB-453 cells, palpable tumors formed by day 10 and large tumors (> 500 $mm^3$) formed within 30 days.

Isolation of the angiogenic sub-population from MDA-MB-415 tumors that switched to the angiogenic phenotype is ongoing. We have developed two new stable breast cancer models and their characterization is ongoing.

Deborah Freedman has studied one of the possible mechanisms of the switch to the angiogenic phenotype. She is working on a hypothesis that certain tumor cells may be able to induce neighboring endothelial cells to bypass senescence and become immortal. To attack this problem she has established a series of immortalized human endothelial cells that overexpress telomerase. She accomplished this by transfecting the gene for telomerase hTert. Increased hTert activity leads to stabilization of telomerase and an unlimited replicated capacity or immortality of the endothelial cells.

During the past year Dr. Freedman has found for the first time a new pathway that may be responsible for the normal senescence of endothelial cells after they have cycled through a predicted number of cell divisions. She found that senescent human endothelial cells contain extremely low or undetectable CDK2 activity which results from the dramatic reduction of CDK2 levels in senescent endothelial cells. She found that CDK2 translation declines during senescence. She showed that bypass of endothelial senescence by telomerase entails the restoration of CDK2 translation and activity. In summary, CDK2 translational downregulation turns out to be a key regulatory event in replicative senescence of endothelial cells.

Understanding the mechanisms which regulate endothelial senescence will be critical in determining the role of endothelial senescence in tumor growth, and whether tumor cells can induce neighboring endothelial cells to bypass senescence.

**Summary of progress in Task 1:**

a. During the past year we have obtained validation that the angiogenic switch is predictable for <u>time</u> and <u>percentage</u> of tumors that switch to the angiogenic phenotype.

b. We have further shown that these angiogenic switching times and percentages of the tumors which undergo the switch are reproducible for a given clone from a single cell.

c. During the next funding year we will address the following questions:

(i) Does antiangiogenic therapy permanently prevent the angiogenic switch after therapy is discontinued?

(ii) If discontinuation of antiangiogenic therapy causes dormancy, does it reset the angiogenic switch to a significantly longer time than the original switching time?

(iii) Gene array analysis will be carried out on the non-angiogenic and angiogenic pairs of each breast cancer to determine if any genes are overexpressed in the non-angiogenic tumors but down-regulated or lost in the angiogenic tumors.

(iv) If so, then genes which are significantly overexpressed only in the non-angiogenic tumors may be transfected into the angiogenic tumors to determine if the angiogenic switch can be suppressed.

**Cooperative studies between Task 1 and Task 2:**

In Task 1 we have ongoing studies that will quantitatively assess the pro- and anti- angiogenic factors in platelet and plasma blood compartments. These studies will elucidate the balance, dynamics, and compartmentalization of pro- and anti- angiogenic factors before, during, and after the angiogenic switch. Mice were injected with the non-angiogenic MDA-MB-436 cells and platelets and plasma were isolated for analysis. Plasma concentrations and platelet loading with pro- and anti- angiogenic factors will be quantified using the ProteinChip system (Series 40000, Ciphergen, Inc.) in collaboration with Dr. Giannoula Klement in Task 3.

We have initiated a detailed study that will dissect the steps of the angiogenic switch in breast cancer. The extent of neovascularization during the angiogenic switch will be quantitatively and qualitatively described at various stages of the angiogenic switch. The MDA-MB-436 cell dormancy model provides us with a unique opportunity for this kind of analysis. We learned the techniques for this approach by visiting Professor Donald McDonald's laboratory at the University of California, San Francisco.

We also have ongoing experiments in collaboration with Randolph Watnick (Task 2) that will assess the effect of thrombospondin-1 on the timing of the angiogenic switch in non-angiogenic tumors. We have infected non-angiogenic breast tumor cells with thrombospondin-1 antisense. We hypothesize

that this endogenous decrease in thrombospondin-1 levels will lead to an earlier switch of non-angiogenic tumor cells to the angiogenic phenotype.

**Task 2: Molecular and genetic studies of the mechanism of the stability of the non-angiogenic phenotype.** (Randolph Watnick, Ph.D.)

Previous results from our group have determined that one form of tumor dormancy is due to an inability of its tumor cells to induce angiogenesis. Our development of a set of non-angiogenic human cancers that remain dormant at a microscopic size, provides an excellent tool to study the regulation of tumor progression as well as the role of the surrounding normal tissue in this process. We hypothesize that the inability of dormant breast tumors to induce neovascularization is due to an imbalance in their production of pro- and anti-angiogenic factors.

We have made the observation that aggressive angiogenic human tumor cells express little to no thrombospondin-1. Thrombospondin-1 is a potent endogenous inhibitor of angiogenesis. Furthermore, the c-Myc oncoprotein, which negatively regulates the expression of thrombospondin-1, is expressed in greater amounts in angiogenic human tumors as compared to non-angiogenic tumor cells.

During the past year we have determined that two breast cancer cell lines that form non-angiogenic, dormant tumors in mice express very high levels of thrombospondin-1 and very low levels of c-Myc or phosphorylated c-Myc. Conversely, we have found that two breast cancer cell lines that form angiogenic tumors in mice, express very low levels of thrombospondin-1 and high levels of c-Myc.

Furthermore, we have determined that angiogenic breast cancer cell lines repress the expression of thrombospondin-1 in surrounding stromal fibroblasts. In contrast, the breast cancer cell lines that produce non-angiogenic dormant tumors actually stimulate thrombospondin-1 expression in stromal fibroblasts. The latter is an unprecedented finding.

We are currently in the process of identifying and purifying the thrombospondin-1 repressing factor(s) that the angiogenic cells secrete, as well as the thrombospondin-1 stimulating factor(s) that the non-angiogenic cells secrete.

We have also found that inhibition of Myc activity in the angiogenic tumor cells results in the loss of thrombospondin-1 activity. Therefore, the expression and/or secretion of the thrombospondin-1 repressing factor(s) appears to be downstream of c-Myc.

**Summary of Task 2 and Next Steps:**

Our working hypothesis is that all breast tumors must pass through the non-angiogenic, dormant phase prior to becoming angiogenic and malignant tumors. Based on our current data we postulate that this transition or "angiogenic switch" is triggered by the increased expression of c-Myc, which, in turn, represses the expression of thrombospondin-1 and allows the tumor to become angiogenic and malignant.

In the next year and beyond we will determine the genetic and molecular mechanisms that regulate the expression of c-Myc in order to determine how the angiogenic switch is triggered.

**Task 3: Development of a novel angiogenesis-based biomarker in platelets to detect non-angiogenic, microscopic-sized dormant tumors before or just after the angiogenic switch, but before tumors can be detected by palpation (i.e., ~50 $mm^3$).**

**Platelet angiogenic profile.** (Judah Folkman, M.D., Giannoula Klement, M.D., & David Cervi, Ph.D.)

Judah Folkman and Giannoula Klement reported to the American Society of Hematology in December 2004 Dr. Folkman's discovery that the proteins contained within platelets shift their concentrations to reflect the angiogenic regulatory proteins elaborated by a tumor.[1] Folkman had previously reported that of the approximately 24 proteins known to be contained within platelets, 13 were positive regulators of angiogenesis and 11 were negative regulators of angiogenesis.[2] He has now shown that these platelet proteins can be accurately quantified by SELDI-ToF mass spectroscopy. We have found that in immunodeficient animals bearing any one of several different types of human cancer, specific angiogenic proteins elaborated by a tumor are taken up by platelets and sequestered. The peak concentration of a given angiogenic protein elaborated by a tumor is in direct proportion to the time the tumor has been present. Therefore, the "platelet angiogenic profile" can detect an early microscopic tumor as small as 1 $mm^3$ or less, and can indicate how long the tumor has been present. For example, if a non-angiogenic tumor is elaborating vascular endothelial growth factor (VEGF), the concentration of VEGF in platelets will increase steadily over time in a linear fashion for as long as 120 days or more. If a human tumor is elaborating more than one angiogenic regulatory molecule, i.e., VEGF, bFGF, PDGF, and endostatin, these will all reveal their increased concentrations in a given platelet sample.

We have developed a standard protocol for sample preparation of platelet lysates. We have developed platelet profile analysis based on the Protein Chip System from Ciphergen, Inc. We now have preliminary data that platelets carry both pro- and anti- angiogenic proteins but exclude other plasma proteins.

Next steps: we are currently conducting a time course experiment that will reveal changes in platelet and plasma profiles of angiogenic regulatory proteins before, during, and after the angiogenic switch. These studies will be performed in both subcutaneously and orthotopically implanted tumors.

**Circulating endothelial cells.** (John Heymach, M.D., Ph.D., Paul Beaudry, M.D., Daniela Prox, M.D.)

Growing evidence now suggests that bone marrow-derived circulating endothelial cells also contribute to tumor neovascularization and tumor growth in some tumors. In collaboration with Dr. Paul Beaudry, we have developed a standard protocol for quantification of circulating endothelial cells in whole blood.

In brief, a cocktail of antibodies is added to a citrated blood sample. These antibodies detect two separate populations of circulating endothelial cells: mature circulating endothelial cells (CECs), and circulating endothelial progenitor cells (CEPs). We have quantitatively compared the systemic mobilization of these two populations of circulating endothelial cells induced by non-angiogenic or angiogenic breast tumors.

We found that at 40 days after subcutaneous inoculation, microscopic non-angiogenic tumors caused a decrease of the CEC population, but angiogenic tumors (~600 $mm^3$ in size) had no effect (see Fig. 2). At the same time point, angiogenic tumors increased the CEP population, but non-angiogenic microscopic tumors had no effect. We have preliminary evidence that the majority of CEC cells originate from the bone marrow, in contrast to a small percentage (~2-3%) of CEP cells. Therefore, even at a microscopic size, non-angiogenic tumors may induce a systemic effect that mobilizes bone marrow CEC cell population. We hypothesize that changes in systemic CEC and CEP populations of cells may be useful as a marker of early cancer.

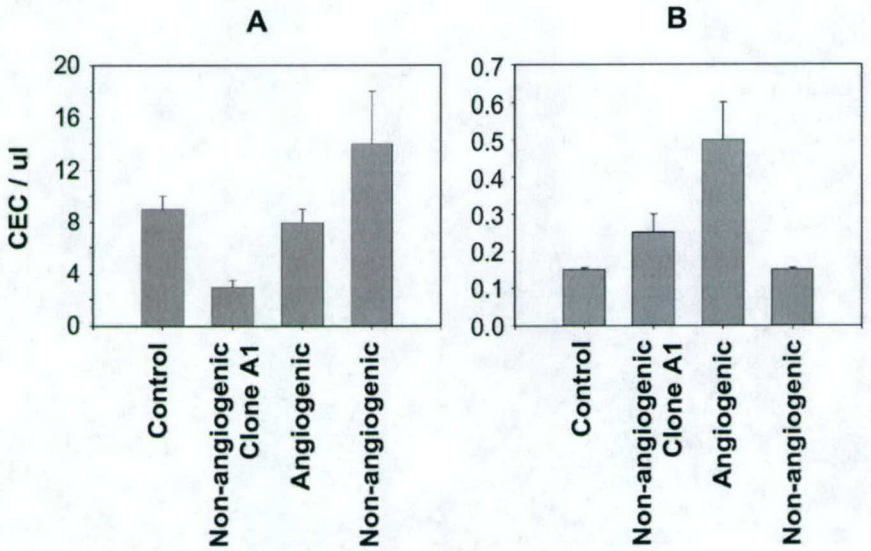



**Figure 2:** Mature circulating endothelial cells (A) and circulating endothelial precursor cells (B) in whole blood analyzed by flow cytometry from mice bearing non-angiogenic and angiogenic tumor cells. (A) Non-angiogenic tumor cells suppress mature circulating endothelial cells (CEC). (B) The angiogenic tumor cells induce mobilization of circulating endothelial precursor cells (CEP) from the bone marrow.

## III. KEY RESEARCH ACCOMPLISHMENTS:

- Established the non-angiogenic subpopulation of four human breast cancers in SCID mice.
- Established reproducible and predictable times to the angiogenic switch for each tumor type.
- Established predictable and reproducible percentages of non-angiogenic tumors for the angiogenic switch for each tumor type.
- Developed a novel biomarker, the "platelet angiogenic profile," which currently may be the most sensitive and most specific biomarker for human cancer available. It can detect the presence of human tumors of less than 1 $mm^3$; it can quantify how long the tumor has been present; and, it can indicate when the angiogenic switch is occurring.
- Continued the development of circulating progenitor endothelial cells as a biomarker of early angiogenic tumors.

## IV: REPORTABLE OUTCOMES:

- The "platelet angiogenic profile" was published in November 2004, and presented in December 2004 as a platform presentation to the American Society of Hematology. Of more than 3,600 papers presented at that meeting, the "platelet angiogenic profile" was among the very few which attracted science writers *(see appendix,* Wall Street Journal *article).*

## V: CONCLUSIONS:

The overall goal of this Innovator Award is to prevent the angiogenic switch in women who face a possible recurrence of breast cancer, or in those at high risk of being diagnosed with the disease.

In **Task I,** the experiments are designed to lead to a clinical application in which non-toxic, FDA approved angiogenesis inhibitors are administered to women before the angiogenic switch occurs in tumors that are still of microscopic size and asymptomatic.

In **Task 2,** our goal is to elucidate the molecular mechanism of the angiogenic switch so that the switch itself could be interrupted, possibly by novel molecules which remain to be discovered. These would be novel types of

angiogenesis inhibitors. This objective is not just wild speculation. It has recently been reported that rosiglitazone, an FDA approved orally available drug for the treatment of type II diabetes, increases the expression of thrombospondin-1 and also increases expression of CD36, the receptor for thrombospondin-1 on endothelial cells.

In **Task 3,** we now have developed a novel biomarker to detect angiogenic regulatory proteins, both positive and negative, elaborated from human breast cancers that are microscopic in size (<1 $mm^3$). This new method called the "platelet angiogenic profile" can detect human cancers in SCID mice before and just after the angiogenic switch. Our next step will be to conduct a time course experiment that will reveal changes in platelet and plasma profiles of angiogenic regulatory proteins before, during, and after the angiogenic switch. These studies will be performed in both subcutaneously and orthotopically implanted tumors. Also planned are studies to determine the sensitivity of this biomarker method by adding VEGF at increasing concentrations (from fentomolar to nanomolar) to platelet rich plasma to determine the lowest concentration of added VEGF that can be detected by our mass spectroscopy.

**"So What Section"**

These studies advance us toward our goal of treating breast cancer at an ultra early stage before it can be located and before it is symptomatic. The goal will be achieved by administering relatively non-toxic angiogenesis inhibitors guided by sensitive and specific angiogenesis-based biomarkers. The biomarkers will be, (i) the platelet angiogenic profile; (ii) circulating endothelial precursors; and (iii) urinary metalloproteinases. Eventually, the first patients may be those women whose breast cancer has been surgically removed. The angiogenesis-based biomarkers could be used periodically (~every three months) to look for recurrence. Any microscopic recurrence may be treated by non-toxic angiogenesis inhibitors until the biomarkers return to normal. The next patients to be studied may be those at high-risk for developing a new primary breast cancer because they harbor a mutated breast cancer gene. Conventional medical practice is to offer these women bilateral mastectomy and bilateral oophorectomy. For those who refuse, we would propose that these individuals could be followed periodically by quantifying their angiogenesis-based biomarkers. Patients with rising biomarkers would be treated by antiangiogenic therapy until the biomarkers returned to normal. Patients whose biomarkers remain normal would not be treated.

As these biomarkers are validated in the clinic over the coming years, it should be possible to extend them to other cancers, such as colon cancer and prostate cancer. We have also recently proposed that it may be possible to raise the level of endogenous angiogenesis inhibitors by administration of small molecular orally available drugs[3] as a novel treatment of ultra early cancer. The long-term goal is to treat cancer before the disease state.[4]

## VI: REFERENCES:


1. Klement G, Kikuchi L, Kieran M, Almog N, Yip T, Folkman J. Early Tumor Detection Using Platelet Uptake of Angiogenesis Regulators. *Blood* 2004; 104:239a, Abstract #839.

2. Folkman J, Browder T & Palmblad J. Angiogenesis research: guidelines for translation to clinical application. *Thrombosis and Haemostasis* 2001; 86:23–33.

3. Folkman J. Endogenous angiogenesis inhibitors. *Acta Pathologica, Microbiologica, et Immunologica Scandinavica* 2004;112:496-507.

4. Folkman J, Kalluri R. Cancer without disease. *Nature* 2004; 427:787.

## VII: APPENDICES:

### I. Pertinent Publications

1. Klement G, Kikuchi L, Kieran M, Almog N, Yip T, Folkman J. Early Tumor Detection Using Platelet Uptake of Angiogenesis Regulators. *Blood* 2004; 104:239a, Abstract #839.

2. Marcus, A. Early Warning: A New Way To Find Cancer. *The Wall Street Journal* 12/07/04.

### II. Personnel Report

### III. Curriculum Vitaes in Biosketch Format

- David Cervi, Ph.D., Research Fellow
- Daniela Prox, Ph.D., Research Fellow
- Sandra Ryeom, Ph.D., Research Associate
- Sarah Short, Ph.D., Research Fellow

*Blood, 104: 239a, Abstract #839, 2004.*

VASCULAR CELL BIOLOGY AND PLATELET ADHESION

**Abstract# 839**

**Early Tumor Detection Using Platelet Uptake of Angiogenesis Regulators.**

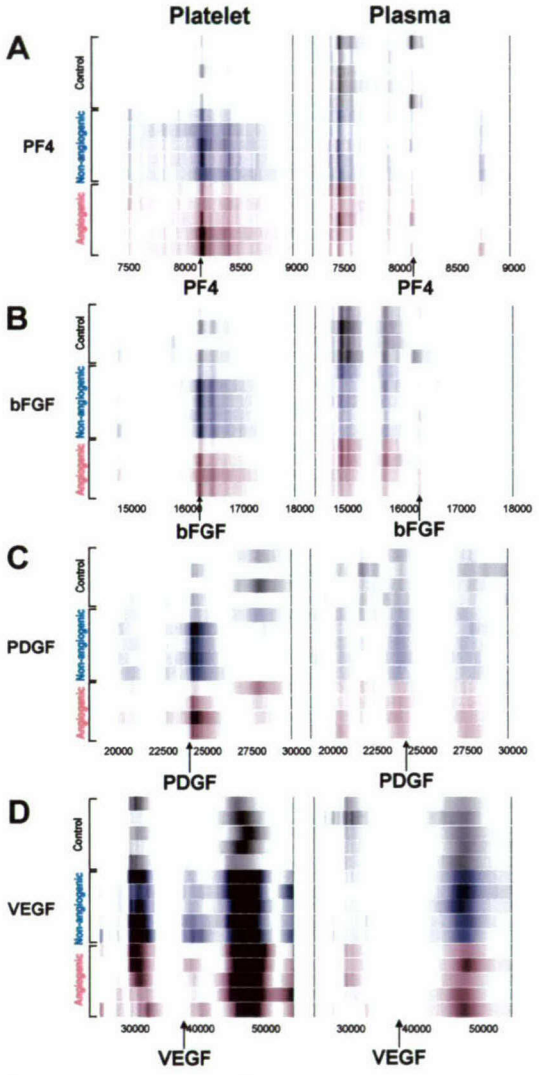
Giannoula Klement,[1,2] Lena Kikuchi*,[1] Mark Kieran*,[2] Nava Almog*,[1] Tai-Tung Yip*,[3] Judah Folkman*.[2] *[1]Vascular Biology Program and Department of Surgery, Childrens Hospital and Harvard Medical School, Boston, MA, USA; [2]Pediatric Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA, USA; [3]Ciphergen Biosystems Inc., Freemont, CA, USA.*

We report a new function for platelets: selective sequestration of tumor-derived angiogenesis regulatory proteins above the concentration of these molecules in plasma.

Iodinated VEGF in a Matrigel pellet (from 100 to 600 ng/ 100 microl), implanted subcutaneously in mice, accumulates almost exclusively in platelets in a dose-dependent manner over a period as long as 2-3 weeks, without raising plasma levels of VEGF. Similarly, platelet VEGF increases in the presence of a single microscopic VEGF-secreting human tumor of up to only 1 $mm^3$ in SCID mice without any increase of VEGF in plasma. In addition to VEGF, other factors such as bFGF, PDGF, BDNF, endostatin and other regulators of angiogenesis are taken up by platelets in a selective and quantifiable manner which is dependent on tumor generation of these molecules. Our data show that these proteins are not simply associated with the platelet surface, but are internalized. Furthermore, they are protected from degradation within the platelet, and are not released by classical degranulating agents, such as thrombin, ADP or epinephrine. Incubation of human platelets with endostatin at above physiological levels results in decrease of the majority of platelet-associated VEGF and bFGF in a concentration-dependent manner.

Using SELDI-ToF mass spectroscopy of platelet extracts, we have found that this novel property of platelets enables the detection of microscopic tumors that undetectable by any presently available diagnostic method. The platelet angiogenic profile is more inclusive than a single biomarker because it can detect a wide range of tumor types and tumor sizes. Relative changes in the platelet angiogenic profile permit the tracking of a tumor throughout its development, beginning from an early *in situ* cancer.

Conclusions: (i) While the half-life of mouse platelets is approximately 3 days, the platelet angiogenic profile persists for as long as the tumor (or Matrigel pellet) is present. This indicates that platelets may continuously scavenge proteins which regulate angiogenesis. (ii) The fact that the presence of a human tumor can now be detected at microscopic size, suggests that it may not be necessary to know the type and location of a tumor before initiating treatment, especially since it is feasible to use anti-cancer therapies of little or no toxicity.




Protein expression maps of platelets and plasma extracts showing differential up-regulation of proteins within the platelets but not in plasma. X-axis is molecular weight of the proteins. Y-axis identifies groups: red are platelet protein extracts collected from mice bearing an angiogenic liposarcoma clone, blue are platelet protein extracts from mice bearing a non-angiogenic liposarcoma clone and grey are platelet protein extracts from control mice without tumors. All platelets samples were collected on day 30 post-tumor implantation. Non-angiogenic tumors are on average 1 $mm^3$ in size, whereas angiogenic average 1 - 2 $cm^3$.

THE WALL STREET JOURNAL.

TUESDAY, DECEMBER 7, 2004

# Early Warning: A New Way To Find Cancer

## *Blood Test Detects Beginnings Of Disease, Suggesting Possibility Of Treatment Before Tumors Grow*

By Amy Dockser Marcus

A GROUP OF researchers say they have found a way to detect cancer earlier, even before the type or the location of the tumor can be known.

The method, which is expected to enter clinical trials early next year, is part of a shift in thinking under way about how early to treat cancer. Long before cancer could ever be detected by current imaging scans or blood tests, the idea is to find an ultra-early indicator of the disease: substances in blood platelets that indicate cancer is in the works. Armed with such information, the researchers involved say, doctors could potentially offer cancer therapies that would prevent tumors from developing—and save patients from having to undergo much more toxic and aggressive treatment once a cancer grows.

While such preventive measure aren't yet being used in the general population, some researchers are already studying the prophylactic use of drugs in patients at very high risk to get cancer.

The platelet substances are a so-called biomarker of cancer—characteristics in bodily fluids that can indicate the presence of disease. Probably the best-known biomarker, prostate specific antigen, or PSA, is related to prostate cancer and is already commonly used. And the search for new and better biomarkers is a hot area of research, with many companies racing to come up with new ones in saliva, urine and blood, among other fluids. The National Cancer Institute earlier this month announced a new round of funding, earmarking $9.8 million to 17 labs looking for cancer biomarkers.

But researchers involved in the platelet study, as well as other cancer experts, say this discovery pushes the issue of early detection further than ever before. In effect, it could allow cancer to be treated similarly to the way doctors now treat heart disease. Just as doctors prescribe statin drugs to help prevent heart attacks in patients with high cholesterol, oncologists could test patients for this early biomarker and prescribe drugs to help prevent cancer. And just as cardiologists don't have to wait for a heart attack in order to act, oncologists wouldn't have to wait until malignant tumors are found.

Drugs in this case would be used to stop the cancerous process itself—rather than targeting a tumor in a particular part of the body. That would be a radical departure from the current approach. "Cancer treatment has always been about location, location, location," says Judah Folkman, director of the vascular biology lab at Children's Hospital and a co-author of the abstract. "If you don't know where the cancer is—if you can't feel it, see it, locate it, or biopsy it—you can't treat it." The marker could also potentially be used to monitor patients who had already had cancer for the earliest signs of recurrence.

Details about the biomarker, developed by researchers at Children's Hospital Boston, the Dana-

*Please Turn to Page D7, Column 3*

### Potential Treatments

Some drugs that may prevent tumors from growing blood vessels:

- Avastin
- Celebrex
- Erbitux
- Herceptin
- Iressa
- Taxol
- Velcade

Source: Judah Folkman, M.D.

# Early Warning: A Way to Find Cancer

*Continued From Page D1*

Farber Cancer Institute in Boston and **Ciphergen Biosystems** Inc., are being presented today at a meeting of the American Society of Hematology. The substances being examined are needed by tumors to grow new blood vessels—the so-called angiogenesis process—which fuels tumors' development.

The usefulness of the marker still needs to be validated in trials involving cancer patients, before it could be used in clinical settings. And some observers note that the cancer biomarkers discovered so far can have problems, such as false positives. Even with the widely used PSA test, doctors still can't tell whether a high reading indicates cancer or a benign condition without doing a biopsy. "Very few biomarkers have really stood up against rigorous testing in a clinical setting," says Sudhir Srivastava, chief of the Cancer Biomarkers Research Group at the NCI.

But researchers on the blood-platelet project believe this biomarker could avoid some of the uncertainties of other markers because it detects substances directly involved in the angiogenesis process in cancer. Giannoula Klement, a pediatric oncologist and the lead author of the abstract, said they plan to launch a trial early next year at Dana-Farber and Children's Hospital in children and adults who either are at very high risk of getting cancer because they have a genetic predisposition, or who have had cancer and may be in danger of recurrence.

William E. Rich, chief executive and president of Ciphergen, which is based in Fremont, Calif., and makes technology involved in studying the platelet substances, said the company "has a very strong interest" in holding clinical trials in other sites around the country as well.

> Blood platelets serve as a natural delivery system inside the body.

The development in recent years of new, less-toxic cancer treatment is one reason that this new idea of preventive therapies may be feasible. So-called smart drugs such as Gleevec, Tarceva, Iressa and Erbitux target only cancerous cells, and have less toxicity than standard chemotherapy regimens. These targeted therapies, which can sometimes be taken for years, have in many cases turned cancer into a manageable chronic disease, similar to the way diabetes is treated. But the drugs have been approved to treat specific cancers, in certain organs, and are given to patients whose tumors are already large enough to be picked up by blood tests or imaging methods.

The potential of finding a cancer biomarker in blood platelets is so significant because it could trigger treatment much, much earlier. Drugs could be used to stop the development of blood vessels in the cancer process itself, before the tumor even has a chance to grow.

It isn't clear yet what drugs would be given in this preventive step. Some existing therapies, such as Avastin and Iressa, are known to block the ability of tumor cells to make certain substances needed to make more blood vessels. And more than 30 drugs that specifically try to inhibit the process of making more blood vessels are working their way through clinical trials to get federal Food and Drug Administration approval. In addition, some drugs used for other conditions, such as the arthritis drug Celebrex, are known to have certain properties that could affect the cancer-development process.

But such efforts are still in very early stages, and more research needs to be done to see that drug treatment offers people the chance of improved survival outcomes. Again, the process has similarities to the treatment of heart disease. Doctors initially gave statins only to patients who had already had heart attacks—and only later used them as a preventive measure once research showed it could work.

Researchers continue to study how the substances in blood platelets contribute to cancer. There are already more than 24 substances in the platelets that have been identified as being part of the angiogenesis process. And Dr. Klement says that platelets serve as a natural delivery system inside the body, collecting at the site of a wound and dumping substances there that are needed to heal a wound. The platelets may be performing a similar function for the tumors, she said, releasing and taking up various proteins the tumor needs to grow.

## Personnel receiving pay from the research effort:

| Name | Role on Project | Annual % Effort |
|---|---|---|
| Judah Folkman, M.D. | Principal Investigator | 30% |
| Nava Almog, Ph.D. | Research Fellow | 50% (03/01/04 - 06/30/04) |
| Amy Birsner | Veterinary Technician | 25% |
| David Cervi, Ph.D. | Research Fellow | 100% (09/01/04 - date) |
| Wendy Foss | Administrative Coordinator | 30% |
| Deborah Freedman, Ph.D. | Research Associate | 100% |
| Lena Kikuchi | Research Technician II | 100% (06/27/04 - 11/13/04)<br>50% (11/14/04 - 01/22/05)<br>40% (01/23/05 - date) |
| Gang Liang, Ph.D. | Research Fellow | 100% (05/10/04 - date) |
| Meghan Lorina | Purchasing Coordinator | 25% |
| George Naumov, Ph.D. | Research Fellow | 20% |
| Daniela Prox, M.D. | Research Fellow | 50% (07/01/04 - date) |
| Frank Rossi | Lab Support Supervisor | 45% (08/22/04 - date) |
| Sandra Ryeom, Ph.D. | Research Associate | 100% (12/15/04 - date) |
| Sarah Schmidt | Administrative Assistant | 20% (09/05/04 - date) |
| Yuen Shing, Ph.D. | Research Associate | 30% (03/01/04 - 03/31/04)<br>25% (04/01/04 - date) |
| Sarah Short, Ph.D. | Research Fellow | 100% (11/01/04 - date) |
| Dessie Stewart | Lab Assistant | 20% (08/22/04 - 09/18/04)<br>15% (09/19/04 - date) |
| Randolph Watnick, Ph.D. | Research Associate | 50% (03/01/04 - 05/31/04)<br>40% (06/01/04 - date) |

## BIOGRAPHICAL SKETCH

| NAME | POSITION TITLE |
|---|---|
| Cervi, David | Research Fellow |
| eRA COMMONS USER NAME | |

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | YEAR(s) | FIELD OF STUDY |
|---|---|---|---|
| University of Windsor, Windsor ON | B.Sc. | 1998 | Biological Sciences |
| University of Windsor, Windsor ON | M.Sc. | 2000 | Cellular Biology |
| University of Toronto, Toronto ON | Ph.D. | 2005 | Molecular & Cellular Biology |

**A. Positions and Honors.**

1998 Laboratory Technician, University of Windsor, Windsor ON
2004 Research Technician, Vascular Biology Program, Children's Hospital, Boston, MA
2005-present Research Fellow, Vascular Biology Program, Children's Hospital, Boston, MA
2005-present Research Fellow in Surgery, Harvard Medical School, Boston, MA

**Honors & Awards**:

1998-2000 Tuition Scholarship (University of Windsor)
1999 Dr. Joseph E. J. Habowsky Graduate Student Teaching Award
2000 Travel Grant American Society for Microbiology
2000-2001 Ontario Graduate Scholarship
2001-2002 Ontario Graduate Scholarship (declined)
2001-2004 University of Toronto Open
2001-2004 Canadian Institute of Health Research

**B. Selected peer-reviewed publications (in chronological order).**

1. Y. Shaked, **D. Cervi**, M. Neuman, L. Chen, G. Klement, C.R. Michaud, M. Haeri, B. Pak, R.S. Kerbel, and Y. Ben-David. (2005) The splenic microenvironment is a source of pro-angiogenesis/inflammatory mediators accelerating the expansion of murine erythroleukemic cells. *Blood* (in press)
2. Y. Shaked, F. Bertolini, S. Man, M.S. Rogers, **D. Cervi**, T. Foutz, K. Rawn, D. Voskas, D.J. Dumont, Y. Ben-David, J. Lawler, J. Henkin, J. Huber, D.J. Hicklin, R.J. D'Amato, and RS. Kerbel. (2005) Genetic heterogeneity of the vasculogenic phenotype parallels angiogenesis: Implications for cellular surrogate marker analysis of antiangiogenesis. *Cancer Cell* **7**:1-11.
3. **D. Cervi,** G. Klement, D. Stempak, S. Baruchel, A. Koki, and Y. Ben-David. (2005) Targeting cyclooxygenase-2 reduces overt toxicity toward low-dose vinblastine and exntends survival of juvenile mice with Friend Disease. *Clinical Cancer Research* (in press).
4. **D. Cervi,** A. Truong, J. Lee, N. Sukhai, Y-J. Li, A. Koki, and Y. Ben-David. (2004) Phosphorylation murine erythroleukemic cells to Celecoxib. *Oncogene* **23**(13):2301-2310.status of c-Kit and Epo receptors, and the presence of wild-type p53 confer in vitro resistance of
5. A.H.L. Truong, **D. Cervi**, J. Lee, and Y. Ben-David. Direct transcriptional regulation of MDM2 by Fli-1: a role for Fli-1 in p53 modulation. (post decision revisions: *Oncogene*)
6. D.E. Spaner, Y. Shi, C. Hammond, J. Mena, **D. Cervi**, A.C. Schuh and L. Radvanyi. Immune-deviation of chronic lymphocytic leukemia-derived dendritic-like cells by serum and anti-oxidants. (post decision revisions: *J Immunotherapy*)
7. Y. Kishi, D. Mahadeo, **D.N. Cervi**, C. Clements, D.A. Cotter and Masazumi Sameshima. (2000) Glucose-induced pathways for actin tyrosine dephosphorylation during *Dictyostelium* spore germination. *Exp. Cell Res.* **256**(1):187-198.

8. D. Cavallo, **D. Cervi**, T. Sands, D.A. Cotter. (1999) Differential *in vitro* activation and deactivation of Cysteine Proteinase isolated from spores and amoebae of *Dictyostelium discoideum*. *Eur. J. Biochem.* **266**(1):132-142

## BIOGRAPHICAL SKETCH

| NAME | POSITION TITLE |
|---|---|
| Prox, Daniela | Research Fellow |

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | YEAR(s) | FIELD OF STUDY |
|---|---|---|---|
| University of Vienna, Austria | | 1997-1998 | Medicine, exchange program |
| Medical School, University of Mainz, Germany | | 1994-2000 | Pre-Medicine and Medicine |
| Medical School, University of Munich, Germany | M.D. | 2000-2001 | Medicine |
| University Children's Hospital, Munich, Germany | | 2002-2004 | Residency in Pediatrics |

### A. Positions and Honors.

1999-2000 Research Technician, Surgical Research, Children's Hospital, Harvard Medical School, Boston, MA

2004-present Research Fellow, Vascular Biology Program, Children's Hospital, Harvard Medical School, Boston, MA

### B. Selected peer-reviewed publications (in chronological order).

Kisker O, Becker CM, Prox D, Fannon M, D'Amato R, Flynn E, Fogler WE, Sim BK, Allred EN, Pirie-Shepherd SR, Folkman J. Continuous administration of endostatin by intraperitoneally implanted osmotic pump improves the efficacy and potency of therapy in a mouse xenograft tumor model. Cancer Res. 2001 Oct 15;61(20):7669-74.

Prox D, Becker C, Pirie-Shepherd SR, Celik I, Folkman J, Kisker O. Treatment of human pancreatic cancer in mice with angiogenic inhibitors. World J Surg. 2003 Apr;27(4):405-11.

## BIOGRAPHICAL SKETCH

| NAME | POSITION TITLE |
|---|---|
| Ryeom, Sandra W. | Instructor/Assistant Professor (paperwork pending) |
| eRA COMMONS USER NAME | |

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | YEAR(s) | FIELD OF STUDY |
|---|---|---|---|
| Wellesley College, Wellesley, MA | B.A. | 1989 | Physics |
| Cornell University, New York, NY | Ph. D. | 1996 | Cell Biology and |
| Harvard Medical School, Boston, MA | Post-Doc | 1997-2004 | Cell Biology |

### A. Positions and Honors

Positions

1989-1991: Research Assistant, Division of Infectious Diseases, Beth Israel Hospital, Boston, MA
1991-1996: Graduate Student, Dept. of Cell Biology and Genetics, Cornell University, NYC, NY
1997-2004: Postdoctoral Fellow: Dept. of Cell Biology Harvard Medical School, Boston, MA
2004-present: Research Associate, Division of Vascular Biology, Dept. of Surgery Children's Hospital
Instructor/Assistant Professor (paperwork pending), Harvard Medical School, Boston, MA

Honors:

1989 Senior Physics Thesis Research Honors, Wellesley College, Wellesley, MA.
1994 Award of Excellence, Vincent Du Vigneaud Symposium, Cornell University, New York, NY.
1994 Trainee Investigator Award, American Federation of Clinical Research, Baltimore, MD.
1994 Selected as member of "Fundamental Issues in Vision Research" course, MBL, Woods Hole, MA.
1997 Recipient of National Reseach Service Award (NRSA) to support post-doctoral research studying the signalling properties of the tight junction protein, ZO-1 in corneal epithelial cells.
2004 Invited speaker, Cold Spring Harbor Protein Phosphatase Meeting, Cold Spring Harbor, NY.

### B. Peer-reviewed publications (in chronological order)


Weller, PF, **Ryeom, SW**, Picard ST, Ackerman, SJ, Dvorak, AM. 1991. Cytoplasmic lipid bodies of neutrophils: Formation induced by cis-unsaturated fatty acids and mediated by protein kinase C. *J. Cell. Biol.* 113:137-146.

Weller PF, **Ryeom SW**, Dvorak AM. 1991. Lipid bodies: structurally distinct, non-membranous intracellular sites of eicosanoid formation. Prostaglandins, Leukotrienes, Lipoxins and PAF. Bailey, J. M., editor. Plenum Press, New York. 353-362.

Dvorak AM, Morgan, E, Schleimer RP, **Ryeom SW**, Lichtenstein LM, Weller PF. 1992. Ultrastructural immunogold localization of prostaglandin endoperoxide synthase to nonmembrane-bound cytoplasmic lipid bodies in human lung mast cells, alveolar macrophages, type II pneumocytes and neutrophils. *J. Histochem. Cytochem.* 40: 759-769.

Fiore, S, **Ryeom SW**, Weller PF, Serhan CN. 1992. Lipoxin recognition sites: specific binding of labeled lipoxin A4 with human neutrophils. *J. Biol. Chem.* 267:16168-176.

**Ryeom SW,** Sparrow JR, Silverstein RL. 1996. CD36 mediates the phagocytosis of rod outer segments by the retinal pigment epithelium. *J. Cell Sci.* 109:387-395.

**Ryeom SW**, Silverstein RL, Scotto A, Sparrow JR. 1996. CD36 interactions with photoreceptor outer segments is mediated by anionic phospholipids. *J. Biol. Chem.* 271:20536-20539.

Sparrow JR,* **Ryeom SW***, Abumrad NA, Ibrahimi A, Silverstein RL. 1997. CD36 expression is altered in retinal pigment epithelial cells of the RCS rat. *Exp. Eye Res.* 64:31-39.

**Ryeom SW**, Paul DL, Goodenough DA. 2000. Truncation mutants of the tight junction protein ZO-1 disrupt epithelial cell morphology. *Mol. Biol. Cell.* 11:1687-1696.

**Ryeom S,** Greenwald, RJ, Sharpe, AH, Mckeon, F. 2003. The threshold pattern of calcineurin-dependent gene expression is altered by loss of the endogenous inhibitor Calcipressin. *Nat. Immunol.* 4:874-883.

(Cited in News and Views) Parry RV and June CH. 2003. Calcium-independent calcineurin regulation. *Nat. Immunol.* 4:821-823.

## BIOGRAPHICAL SKETCH

| NAME | POSITION TITLE |
|---|---|
| Short, Sarah | Research Fellow |

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | YEAR(s) | FIELD OF STUDY |
|---|---|---|---|
| University of California, Santa Cruz, CA | B.A. | 1985 | Chemistry |
| University of North Carolina, Chapel Hill, NC | Ph.D. | 1999 | Pharmacology |

**A. Positions and Honors.**

**Professional Experience**

1983-1985 Research Assistant, UC Santa Cruz, Santa Cruz, CA
1985-1991 Biochemist, SRI International, Menlo Park, CA
1991-1994 Research Associate I, Genentech, Inc., S. San Francisco, CA
2000-present Research Fellow, Children's Hospital, Harvard Medical School, Boston, MA

**Awards**

1989 Genetic Environmental Toxicological Association outstanding presentation award
1989 SRI outstanding performance award
1992 Genentech award for significant contribution to research project
1994 Hoechst-Celanese Scholarship award (UNC-CH research excellence award)

**B. Selected peer-reviewed publications (in chronological order).**


1. Baker, H., G. J. Handelman, S. Short, L. J. Machlin, H. N. Bhagavan, E. A. Dratz and O. Frank. (1986). Comparison of plasma alpha- and gamma-tocopherol levels following chronic oral administration of either all-rac-α-tocopheryl acetate or RRR- α- tocopheryl acetate in normal adult male subjects. *Amer. J. Clin. Nutr.* 43:382-387
2. Stephens, R. J., D. S. Negi, S. M. Short, F. J. G. M. van Kuijk, E. A. Dratz and D. W. Thomas. (1988). Vitamin E distribution in ocular tissues following long-term dietary depletion and supplementation as determined by microdissection and gas chromatography-mass spectroscopy. *Exp. Eye Res. 47:237-245*
2. Stephens, R. J., D. S. Negi, S. M. Short, F. J. G. M. van Kuijk, E. A. Dratz and D. W. Thomas. (1988). Lipid peroxidation and retinal photoxic degeneration. *Basic Life Sci. 49:283-9*
3. Handelman, G. J., P. Kosted, S. Short and E. A. Dratz. (1989). Determination of selenium in human blood by high performance liquid chromatography with fluorescence detection. *Anal.Chem. 61:2244-2249*
4. Knadle, S. A., C. E. Green, M. Baugh, M. Vidensek, S. M. Short, X. Pardos and C. Tyson. (1990). Trichlorethylene biotransformation in human and rat primary hepatocytes. *Toxic. In Vitro. 415:537-541*
5. Murphy, B. J., K. R. Laderoute, S. M. Short and R. M. Sutherland. (1991). The identification of heme oxygenase as a major hypoxic stress protein in chinese hamster ovary cells. *Br. J. Cancer, 64:69-73*

6. Laderoute, K. R., B. J. Murphy, S. M. Short, T. D. Grant, A. M. Knapp, and R. M. Sutherland. (1992). Enhancement of transforming growth factor-alpha synthesis in multicellular tumor spheroids of A431 squamous carcinoma cells. *Br. J.Cancer. 65:157162*
7. Short, S. M., W. Rubas, B. D. Paasch, and R. J. Mrsny. (1995) Transport of biologically active interferon-gamma across human skin *in vitro. Pharm. Res. 12:1140-1145*
8. Mrsny, R. J., A. L. Daugherty, S. M. Short, R. Widmer, M. W. Siegel, and G-A. Keller. (1996). Distribution of DNA and alginate in purulent cystic fibrosis sputum: implications of pulmonary targeting strategies. *J. Drug Targeting, 4:233-243*
9. Short, S. M., B.D. Paasch, N. Weiner, and R. J. Mrsny. (1996). Percutaneous absorption of biologically-active interferon-gamma in a human skin-graft nude mouse model. *Pharm. Res. 13:1020-1027*
10. Short, S. M., G. L. Talbott, and R. L. Juliano. (1998). Integrin-mediated signaling events in human endothelial cells. *Mol. Biol. Cell 9:(8) 1969-1980*
11. Delong, R.K., H. Yoo, S.K. Alahari, M. Fisher, S.M., Short, S.H., Kang, R. Kole, V. Janout, S.L. Regan, and R.L. Juliano. (1999). Novel cationic amphiphiles as delivery agents for antisense oligonucleotides. *Nucleic Acids Res. 27(16):3334-41*
12. Aplin, A. E., S. M. Short, and R. L. Juliano. (1999). Anchorage-dependent regulation of the mitogen-activated protein kinase cascade is supported by a variety of integrin alpha-chains. *J. BioL Chem. 274 (44): 31223-8*
13. Short, S. M., J. L. Boyer, and R. L. Juliano. (2000). Integrins regulate the linkage between upstream events in G protein-compled receptor signaling to mitogen-activated protein kinase. *J. Biol Chem. 275 (17):12970-7*
14. Juliano, R. L., A. E. Aplin, A. K. Howe, S. Short, J. W. Lee, and S. Alahari. (2001). Integrin regulation of receptor tyrosine kinase and G protein-coupled receptor signaling to mitogen-activated protein kinases. *Methods in Enz.* 333:151-63.
15. Furman, C., S. M. Short, R. R. Subramanian, B. R. Zetter, and T. M. Roberts. (2002). DEF-1/ASAP1 is a GTPase-activating protein (GAP) for ARF1 that enhances motility through a GAP-dependent mechanism. *J. Biol. Chem.* 277(10):7962-9.
16. Short, S. M., A. Derrien, R.P. Narsimhan, J. Lawler, D.E. Ingber, and B.R. Zetter (2005). Inhibition of endothelial cell migration by thrombospondin-1 type-1 repeats is mediated by Beta-1 integrins. *In Press, J. Cell Biol.*
17. Satchi-Fainaro, R., R. Mamluk, L. Wang, S. M. Short, D. Feng, A. M. Dvorak, H. F. Dvorak, M. Puder, D. Mukhopadhyay and J. Folkman. (2005) Inhibition of vessel leakiness by TNP-470 and its polymer conjugate *In Press, Cancer Cell*